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Quality by design (QbD) approach to formulate *in situ* gelling system for nose to brain delivery of Fluoxetine hydrochloride: *Ex-vivo* and *In-vivo* study

Enfoque de calidad por diseño (QbD) para formular el sistema de gelificación *in situ* para el suministro desde la nariz al cerebro del hidrocloruro de fluoxetina: estudio *in vitro* e *in vivo*

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RESUMEN

Objetivo: El propósito del presente estudio es desarrollar un gel nasal sensible a iones de clorhidrato de Fluoxetina (FXH) para el suministro cerebral. Se utilizó un diseño factorial 32 para investigar el efecto de la variable independiente en las variables dependientes.

Métodos: Se evaluaron las formulaciones para el estudio de gelificación, viscosidad, resistencia de gel, fuerza de mucoadhesión, contenido de fármaco, permeabilidad in vitro de fármacos, estudio farmacodinámico in vivo y estudio de estabilidad.

Resultados: Los resultados revelaron que a medida que aumentaba la concentración de goma de gelano y HPMC, había un incremento en la viscosidad y resistencia a la mucoadhesión y disminución en el porcentaje de liberación. La formulación F4 optimizada mostró la liberación de fármaco más alta del 94,24%. En el estudio de actividad locomotora y prueba de natación forzada, las ratas tratadas con gel in situ mostraron respuestas significativas en comparación con el grupo de control. Los exámenes histopatológicos no mostraron evidencia de daño en la mucosa nasal. El gel nasal in situ fue estable después de 3 meses.

Conclusión: Se concluyó que las formulaciones nasales de clorhidrato de fluoxetina que mejoraban la absorción nasal y la conformidad del paciente para el tratamiento de la depresión.

Palabras clave: Absorción nasal, clorhidrato de Fluoxetina, Farmacodinámico

ABSTRACT

Objective: The purpose of the present study is to develop an ion sensitive *in situ* nasal gel of Fluoxetine hydrochloride for brain delivery. A 3^2 factorial design was used to investigate effect of independent variable on dependent variables.

Methods: Formulations were evaluated for gelation study, viscosity, gel strength, mucoadhesion strength, drug content, *ex-vivo* drug permeation, *in vivo* pharmacodynamic and stability study.

Results: The results revealed that as the concentration of gellan gum and HPMC were increased, there was increase in viscosity and mucoadhesive strength and decrease in percent release. The optimized formulation F4 showed highest drug release 94.24 %. In locomotor activity and forced swim test study, the *in situ* gel treated rats showed significant responses as compared to control group. Histopathological examinations showed no evidence of nasal mucosal damage. The *in situ* nasal gel was stable after 3 months.

Conclusion: It was concluded that, the *in situ* nasal formulations of Fluoxetine hydrochloride which enhanced nasal absorption and patient compliance for the treatment of depression.

Keywords: Nasal, Fluoxetine hydrochloride, Pharmacodynamic

INTRODUCTION

Depression is a common psychiatric disorder, and statistics clearly identify it as a major public health problem[1]. The major disadvantage with conventional dosage forms are many patients find it difficult to swallow (dysphagia) tablets and capsules[2]. Nose to brain delivery has number of advantages, such as drugs can be rapidly absorbed through the nasal mucosa, giving rapid onset of action. The olfactory region of nasal mucosa that provides a direct connection between nose and brain can be exploited for targeting central nervous system (CNS)-acting enormous range of drug molecules of neurotherapeutics, both macromolecules and low molecular weight drugs, used in conditions such as Alzheimer's disease, depression, migraine, schizophrenia[5]. Both hydrophobic drugs, e.g. propranolol[7] and hydrophilic drugs, e.g. vanlafaxine hydrochloride[8] are absorbed by the nasal mucosa. The gellan gum is an ion sensitive polymer which forms clear gel in contact with monovalent and divalent cations. In an ion free aqueous medium, it forms double helices which at room temperature are only weakly attached to each other (van der Waals attraction). In the presence of cations some of the helices associate into aggregates and cause cross-linking of the polymer chains[9]. Fluoxetine hydrochloride (FXH) is a selective serotonin reuptake inhibitor as an antidepressant, is widely used to treat various types of psychiatric disorders[1011]. The dose of FXH ranges from 20 to 80 mg administered as 1 to 4 capsules a day. This dose and frequency may cause enhanced drug-related side effects and may pose compliance problems[12]. The long half-life of fluoxetine and its active metabolite essentially preclude a withdrawal phenomenon. It is an inhibitor of cytochrome P450 (CYP)2D6 and other CYP enzymes, which increases the potential for drug interactions and also undergoes extensive hepatic first-pass metabolism[13-15]. The objective of the present work was to prepare *in situ* gel of FXH for enhanced drug absorption in brain through nasal route and patient compliance for the treatment of depression.

MATERIALS AND METHODS

Materials

(FXH) was obtained from Swapnaroop drugs and chemicals, Aurangabad. Gellan gum (Gelrite) was procured as gift samples from Signet Chemical Corporation Ltd. H*ydroxypropyl methylcellulose* (HPMC) were obtained as a gift sample from Colorcon, Mumbai, India. Mannitol and propyl paraben were obtained from Molychem India, Mumbai.

Methods

In the present study 3^2 full factorial designs (Design Expert 7.0.0.0) was selected to obtain nine formulation and to study the effect of independent variables gellan gum (X_1) and HPMC (X_2) on dependent variables such as viscosity, mucoadhesive strength and drug release.

Preparation of *in situ* gel

Gellan gum and HPMC was weighed and dispersed separately in distilled water and stirred by mechanical stirrer (Remi motors Ltd, Mumbai, India, type RQ-122) for 30 min at 90°C in a water bath and then cooled to room temperature. FXH (1% w/v) and HPMC solution was added in gellan gum solution slowly with continues stirring. Appropriate quantities of mannitol as a osmotic agent and propyl paraben were added simultaneously [16]. The compositions of prepared formulations of FXH are shown in (Table 1).

Table 1: The compositions of prepared formulations of FXH

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Fluoxetine HCl (%)	1	1	1	1	1	1	1	1	1
Gellan gum (%)	0.2	0.2	0.2	0.4	0.4	0.4	0.6	0.6	0.6
HPMC (%)	0.1	0.15	0.2	0.1	0.15	0.2	0.1	0.15	0.2
Mannitol (%)	4	4	4	4	4	4	4	4	4
Propyl paraben (%)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Distilled water (q.s) ml	100	100	100	100	100	100	100	100	100

Gelation study

The gelation study was done by taking formulation and simulated nasal fluid SNF (1:1v/v ratio) and mixed on magnetic stirrer (Magnetic stirrer, Remi). The gelation point was determined when the magnetic bar stopped moving due to gelation[17]. The consistency of formed gel was checked and graded, as indicated in (Table 2).

Viscosity study

The viscosity of nasal formulation before and after gelation was determined using Brookfield Rheometer R/S-CPS +1600 (Lauda Ecoline Staredition RE-204). Each point is the average of at least three readings[18].

Gel strength study

Test was performed using a gel strength apparatus modified at the laboratory. *In situ* gel formulation (50 g) was placed in a 100 ml measuring cylinder and gelation was induced by simulated nasal fluid. The apparatus for measuring gel strength (weight: 35 g) was then placed on the gel. The gel strength was measured by the minimal weight that pushed the apparatus 5 cm down through the gel[19].

Mucoadhesive strength study

Fresh nasal mucosa was obtained from the local slaughter house (Aurangabad), was carefully removed from the nasal cavity of sheep and mounted on glass surface using adhesive tape while another mucosal section was fixed in inverted position to the cylinder. 50 mg of gel was placed on mucosal surface. The glass mounted mucosal surface with gel formulation and mucosal surface attached to cylinder were held in contact with each other for 2 min to ensure intimate contact between them. In second pan, the weights were increased until the two mucosal tissues got detached from each other. The nasal mucosa was changed for each measurement. The mucoadhesive force expressed as the detachment stress in dynes/cm^2 was determined from the minimal weight that detached the mucosal tissue from surface of each formulation[20].

$$\text{Mucoadhesive strength (dynes/cm}^2) = mg/A$$

Where,

m = Weight required for detachment in gram,

g = Acceleration due to gravity (980 cm/s^2),

A = Area of mucosa exposed.

Drug content

The drug content was determined by taking 1ml of formulation in a 50 ml volumetric flask diluted with phosphate buffer pH 6.6 and analyzed using UV-visible spectrophotometer (Shimadzu, UV-1800, Lab India) at λ_{max} 263.80 nm.

Ex-vivo permeation study

Ex-vivo permeation study of formulations was carried out using Franz diffusion cell. Nasal mucosa was placed in diffusion cells displaying a permeation area of 0.785 cm^2. The receiver compartment containing phosphate buffer pH 6.6 as in range of nasal cavity pH was maintained at 37 ±0.5°C. After a pre-incubation time of 20 min, formulation equivalent to 10 mg of FXH was placed in the donor chamber containing 3 ml of artificial nasal fluid. At predetermined time points (30, 60, 120, 180 and 240 min), 1ml of sample was withdrawn from the receptor compartment, replacing with fresh medium[21]. The amount of drug permeated was determined using UV-visible spectrophotometer (Shimadzu, UV-1800, Lab India) at λ_{max} 263.80 nm.

Release mechanism

To study the mechanism of drug release from prepared gelling system, the in vitro permeation data were fitted to zero order, first order, Higuchi release model, Hixson and Crowell method and Korsemeyer-Peppas model by using DD Solver software, and the model with the higher correlation coefficient was considered to be the best model[22].

Histopathological study

Histopathological examination on control mucosa and F4 formulation treated mucosa was performed using a light microscope (Nikon Eclipse E600, Japan). Tissue was fixed with 10% buffered formalin (pH 7.0), routinely processed and embedded in paraffin. Sections (5µm) were cut on glass slides and stained with hematoxylin and eosin.

Stability study

The stability study as per ICH guideline on optimized formulation F4 was carried out at, temperature 40^0 C ± 2 °C and humidity 75% RH ± 5% condition in stability chamber (HMG, India) for three months. The formulation was examine for pH, drug content, viscosity[23].

In vivo pharmacodynamic study

IAEC, M.E.S. College of Pharmacy, Sonai approved the protocol for *In vivo* pharmacodynamic study. Locomotor

activity test (LAT) and Forced swim test (FST) was carried out to evaluate the antidepressant effect of the optimized F4 formulation. Rats of either sex weighing 250–300 g were selected and kept under standard laboratory conditions (temperature 23-30^{0}C) with free access to standard laboratory diet. Rats were divided randomly into three groups, each containing six animals (n= 6). Group one was treated with saline and was considered as a control. Group two was treated with oral tablets of FXH containing dose of 10 mg/kg. Group three was treated with optimized F4 formulation through nasal containing 10 mg/kg. The doses were administered without anesthesia by using simple poly-ethylene tube.

Force swim test

The tank was filled 90 % with water so they swim without touching their hind limb or tail to the bottom of the tank. On day one of experiments, rats were forced to swim for 10 min. After 24 h, rats were re-exposed to forced swim after administration of dose for 5 min and animals were judged for immobility, climbing, and swimming and experiments were performed for thrice. After experiment, the rat was removed from the tank and returned to their cage [24]

Locomotor activity test

Locomotor activity was measured in the open-field test. The apparatus consisted of a square arena (200×200 cm), with a 50 cm height. The floor was divided into 30 equal squares. Animals were individually positioned in the center of the arena and the activity was measured over 5 min (numbers of square crossed). A square crossed was defined as the rat placing its four paws into the quadrant and going to the neighboring quadrant. The open field was cleaned with isopropyl alcohol solution before behavioral testing to avoid possible bias due to odors and/or residues left by rats tested earlier. Also, after each 3 animals apparatus was cleaned[25].

RESULTS AND DISCUSSION

Analysis of data

Statistical model incorporating interactive and polynomial terms was utilized to evaluate the responses.

$$Y=b0+b1X1+b2X2+b12X1X2+b11X_1^2+b22X_2^2$$

Where, Y is the dependent variable, b0 is the arithmetic mean response of the nine runs and b1 (b1, b2, b12, b11 and b22) is the estimated coefficient for the corresponding factor X1 (X1, X2, X12, X11 and X22), which represents the average results of changing one factor at a time from its low to high value. The interaction term (X1X2) depicts the changes in the response when two factors are simultaneously changed.

$$Y \text{ (Viscosity)} =66.44+9.57X_1+27.93X_2$$

From above equation the positive coefficient of variable X_1 and X_2 indicate that, as concentration of gellan gum and HPMC increased the viscosity was also increased.

$$Y(\text{Mucoadhesive strength}) =+2564.26+208.89\ X_1+290.78\ X_2-5.25\ X_1\ X_2+80.45\ X_1^2\ 4.89\ X_2^2$$

From above equation the positive coefficient of variable X_1 and X_2 indicate that, as concentration of gellan gum and HPMC increased the mucoadhesive strength was also increased.

$$Y(\%\text{ drug release}) = +86.88 -2.40\ X_1-6.95\ X_2$$

From above equation the negative coefficient of combine variable X_1 and X_2 indicate that as concentration of gellan gum and HPMC increased the percent drug release decrease.

Response surface plot (RSP)

From RSP evaluation it was found that concentration of gellan gum and HPMC increased, mucoadhesive strength and viscosity increases. Where as in case of drug release concentration of polymer increases the permeation release decreases as shown in Figure 1. The RSP results were similar to the mathematical data.

Gelation study

In gelation study, it was observed that as concentration of gellan gum increased (0.2 to 0.6 %) gelation point increased. The formulation F1 to F3 showed less gelation point whereas F4 to F6 showed immediate gelation remain for few hours (less stiff gel). Further as concentration of gellan gum increased the immediate gelation remain for extended period (stiff gel). Gelation was assessed on a scale ranging between - and +++, as shown in (Table 2). From above results, it revealed that the HPMC did not affect the gelation phenomena, but the gellan gum plays a critical role in hydrogel formation.

Viscosity study

In viscosity study it was found that as concentration of gellan gum and HPMC increased, the viscosity goes on increasing. Formulation F1 to F9 the concentration of HPMC increased (0.1 to 0.2%) and gellan gum (0.2 to 0.6 %), the viscosity increased before and after gelation as shown in Table 2.

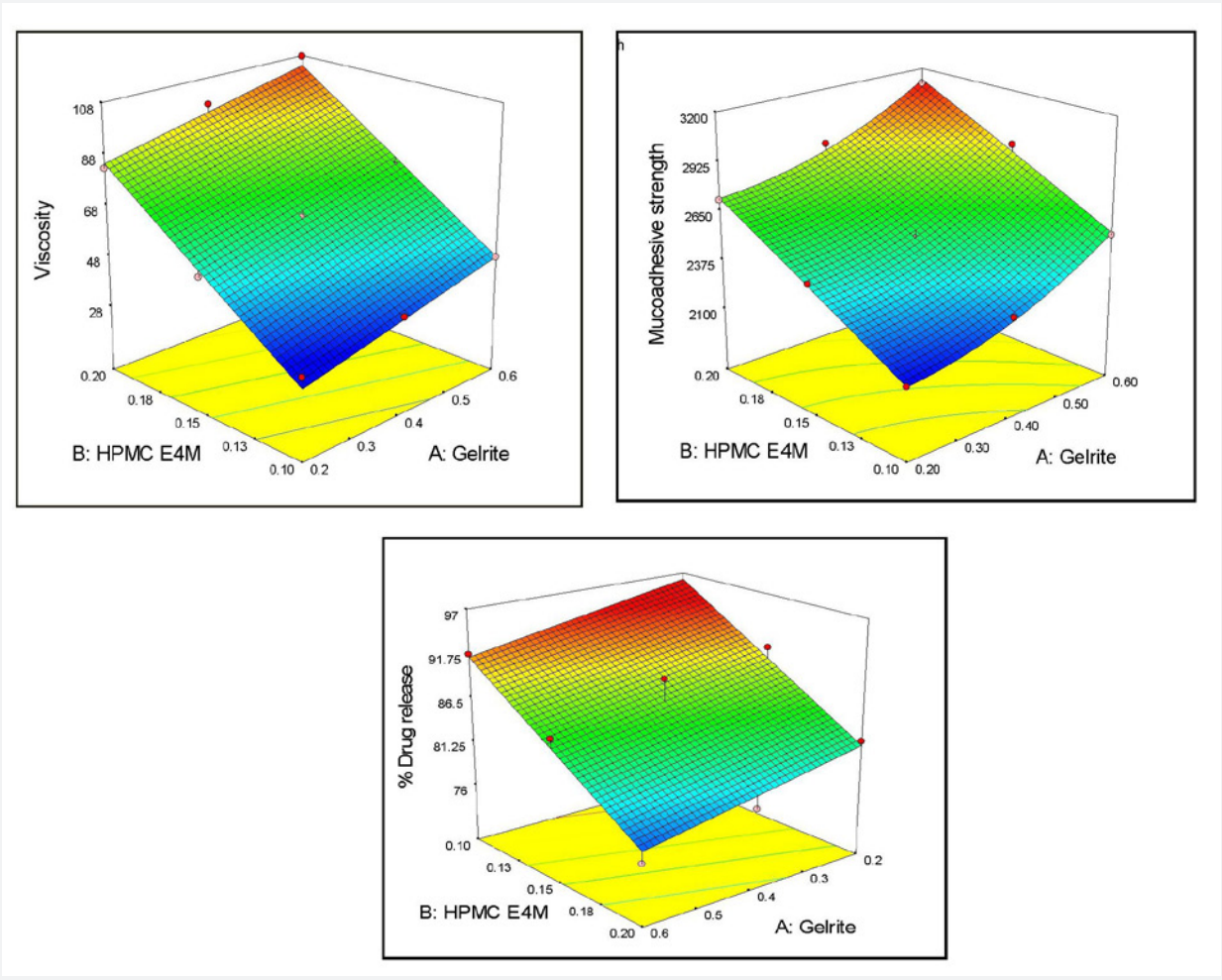

Figure 1. Response surface plot for viscosity, mucoadhesive strength and drug release

Gel strength

The results of all formulations had gel strength between 18.67 sec to 56.67 sec as shown in (Table 2). The formulation F1 and F2 showed the less gel strength and may not retain its integrity and erode rapidly, whereas F3 to F7 show the gel strength in range 27.33 sec to 47.67 sec, remain stable and retain its integrity. In formulation F8 and F9 showed gel strength more than 50 sec which may form stiff gel and caused discomfort to nasal mucosa. Gel strength above 50 seconds suggests undesirable stiffness of gel formulation which may lead to irritation and discomfort in drug delivery. The gel strength values ranging 25-50 sec are considered adequate [26].

Mucoadhesive strength study

Study indicates that, the concentration of HPMC increases the mucoadhesive strength goes on increasing shown in (Table 2). HPMC is a hydrophilic polymer with many polar functional groups. Upon hydration the polymeric chains of HPMC get entangled with glycoprotein chains of mucin resulting in bioadhesion [2728].

Drug content

The drug content was in the range 98.03- 101.07 %, which revealed that FXH was uniformly dispersed in gelling system. The results are shown in (Table 2).

Table 2: Drug content, gelling capacity, viscosity, gel strength and mucoadhesive strength

Formulation code	Gelling capacity	Viscosity (cp)		Gel strength (sec)	Mucoadhesive strength (dynes/cm^2)	Drug content (%)	% Drug permeated
		Sol	Gel				
F1	+	33.16±0.3	101.43± 2.25	18.67±0.58	2135±2.65	98.67±0.53	80.52±3.2
F2	+	39.06±0.6	112.198± 5.86	24±1.0	2446.67±2.08	98.03±0.73	74.50± 4.5
F3	+	47.80±0.3	124.683±8.52	27.33±0.58	2716±3.46	99.28±0.29	70.02± 5.8
F4	++	54.35±0.2	143.39±7.52	31±1.0	2282±2.65	99.79±0.29	94.24±2.6
F5	++	63.67±0.5	164.99± 8.45	34±1.0	2515.33±0.58	100.16±1.10	85.52±5.4
F6	++	72.31±0.5	182.82± 5.23	42.33±1.53	2885.67±2.52	99.66±1.25	80.12±3.4
F7	+++	82.75±0.3	197.71± 8.52	47.67±2.08	2549.67±2.08	101.07±0.36	82.18±4.5
F8	+++	97.26±0.3	220.43± 6.24	52.33±0.58	2891.67±1.53	99.13±0.29	80.12±5.4
F9	+++	107.60±0.8	237.15± 10.23	56.67±2.08	3109.67±2.52	99.80±0.25	75.10±5.7

Ex-vivo permeation study

The results reveal that as the concentration of gellan gum (0.2 to 0.6%) and HPMC (0.1 to 0.2 %) increased, the drug release was decreased. The percentage drug permeated after 240 min from all formulations was found to be between 75.10 to 94.24%. The highest drug release was found in F4 formulation (94.24%), which was selected as optimum formulation for further study. Permeation profiles for all formulations are shown in (Figure 2). The initial rates of permeation were very rapid due to incomplete gel formation, but as the time progress the permeation rate decreases due to complete gel formation. With an increase in concentration of HPMC (0.1 to 0.2 %), the diffusion rates were found to decrease gradually. It was proposed that as the concentration of gellan gum increased, the polymer chains approached closer, and the number of interactions between the polymer chains increases which leads to a denser 3-D network structure[29]. Decreased in drug released might be due to the increase in polymer concentration which increases the viscosity of gel layer with longer diffusional path length, resulting in greater retardation of drug in gel.

Release mechanism

The results obtained from release kinetics it could be concluded that, the formulations (F1 to F9) exhibited n values between 0.542–0.986 indicating an anomalous or nonfickian release suggesting a coupled erosion– diffusion mechanism.

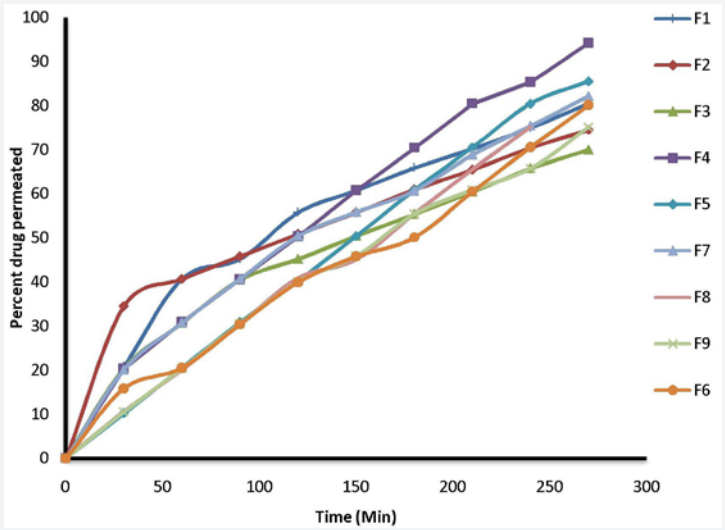

Figure 2. Permeation profile of all formulations through sheep nasal mucosa

Histopathological study

Intact columnar structure of epithelial cell was observed in both nasal mucosa (treated and untreated) shown in Figure 3A. From results it was found that prepared formulation is safe to use.

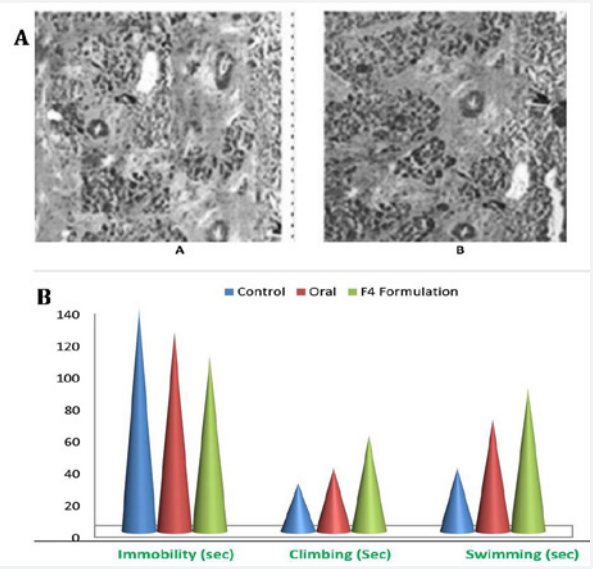

Figure 3.
A). Histopathological image of sheep nasal mucosa A-treated, B- untreated. B). Results of forced swim test.

In vivo pharmacodynamic study

Force swim test

The FST is most used tool for screening antidepressants activity[24]. The F4 administer nasal route reduced total immobility period and increase climbing and swimming behavior as compared to control, orally administer group as shown in Figure 3B. This might be due to the required amount of FXH available in brain through nasal route.

Locomotor activity study

In study, difference in the values was found in control, orally administer and F4 formulation treated group through nasal as shown in Table 3. The results revealed that animals were not hyperactive.

Stability Study

In stability study there was no change found in drug content, pH and viscosity as shown in (Table 4). From stability study results, it was concluding that prepared formulation is stable.

Table 3: Stability results of optimized F4 formulation

Days	Drug content (%)	pH	Viscosity (cp)
0	99.79±0.29	5.81±0.02	54.35±0.24
30	99.34±0.11	5.80±0.06	56.12±0.09
60	99.03±0.06	5.80±0.09	57.39±0.17
90	98.87±0.13	5.78±0.15	58.60±0.11

Table 4. Results of locomotor activity.

Treatment group	No of square crossed			Mean ± SD, n = 3
Control	74±2	78±4	76±2	77±2
F4 Formulation	86±3	87±3	86±3	87±2*
Oral administration	85±2	84±3	85±2	84±3
Values are expressed in mean ± SD, n = 3 , *p value < 0.05 considered statistically significant compared to control				

CONCLUSION

A 3^2 factorial design was used successfully to study the effect of different variables. The optimized formulation F4 containing 0.4% gellan gum and 0.1% HPMC showed highest drug release (94.24 %) through sheep nasal mucosa. In pharmacodynamic study, the *in situ* gel treated rats showed significant responses as compared to control group. It was concluded that, the *in situ* nasal formulations of FXH was effectively formulated using quality by design approach which enhanced drug absorption in brain through nasal route and patient compliance for the treatment of depression.

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